Published in final edited form as:
Pharmacogenomics J. 2007 December ; 7(6): 368–379.

Nucleotide sequence variation within the human tyrosine kinase B neurotrophin receptor gene (*NTRK2*): association with antisocial alcohol dependence

K. Xu, **T. R. Anderson**, **K. M. Neyer**, **N. Lamparella**, **G. Jenkins**, **Z. Zhou**, **Q. Yuan**, **M. Virkkunen**[1], and **R. H. Lipsky**[*]
Sections on Molecular Genetics and Human Genetics, Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892

1 *Department of Psychiatry, University of Helsinki, Finland*

Abstract

To identify sequence variants in genes that may have roles in neuronal responses to alcohol, we resequenced the 5′ region of *NTRK2* and determined linkage disequilibrium (LD) values, haplotype structure, and performed association analyses using 43 single nucleotide polymorphisms (SNPs) covering the entire *NTRK2* region in a Finnish Caucasian sample of 229 alcohol dependent subjects with antisocial personality disorder and 287 healthy controls. Individually, three SNPs were associated with alcohol dependence and alcohol abuse (AD)(*P*-value from 0.0019 to 0.0059, significance level was set at $P \leq 0.01$ corrected for multiple testing), while a common eighteen-locus haplotype within the largest LD block of *NTRK2*, a 119 kb region containing the 5′ flanking region and exons 1 through 15, was marginally overrepresented in control subjects compared to AD individuals (global $P = 0.057$). Taken together, these results support a role for the *NTRK2* gene in addiction in a Caucasian population with AD and a subtype of antisocial personality disorder.

Keywords

alcoholism; gene; tyrosine kinase B receptor; TrkB; *NTRK2* gene; single nucleotide polymorphism; SNP; association

Introduction

Alcohol dependence (MIM 103780) is a common complex addiction having a heritability of at least 50%, in both men and women.[1] Approximately 7 % of the adult U.S. population (about 18 million individuals) is diagnosed with alcohol abuse or dependence.[2] Vulnerability to alcoholism is most likely due to multiple interacting genes, each having a modest contribution to the phenotype and gene-environment interactions. Understanding the contribution of allelic variation to alcohol dependence (AD) will aid in defining neurochemical pathways that are altered in the brain to produce addiction and to better understand mechanisms that may be shared with other habit-forming behaviors in humans, such as cigarette smoking.[3]

*To whom correspondence should be addressed: Robert H. Lipsky, Ph.D., NIAAA, NIH, 5625 Fishers Lane, Room 3S32, Rockville, MD 20852, Tel: 301-402-5591, Fax: 301-480-2839, E-mail: rlipsky@mail.nih.gov.

Web Resources
The URLs for data presented herein are as follows: dbSNP, http://www.ncbi.nlm.nih.gov/SNP
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim HapMap Project, http://www.hapmap.org

Candidate gene studies and linkage-based genome scans have identified multiple chromosomal regions as sources of potential susceptibility to AD as well as other addictions, showing some convergent findings.[4] Examples of this convergence are the genes encoding alcohol dehydrogenase IB and aldehyde dehydrogenase 2, which were originally based on the mechanism of the association of ADH1B and ALDH2 polymorphisms with AD in that the isoenzymes encoded by these alleles lead to an accumulation of acetaldehyde during alcohol metabolism. In other investigations of interest to the current study, cigarette smoking with or without AD, were linked to broad regions of chromosomes 9 and 11.[4–9] Among the candidate addiction susceptibility genes defined by these linkage signals were those that encode the neurotrophin, brain-derived neurotrophic factor (*BDNF*, chromosome 11), and its cognate receptor, neurotrophic tyrosine kinase receptor B (TrkB) (*NTRK2*, chromosome 9).

Neurotrophins are a family of developmentally regulated proteins that are critical for differentiation and survival of post-mitotic neurons and in synaptic plasticity. The biological activity of neurotrophins is mediated by the Trk family of receptors having intrinsic protein-tyrosine kinase activity[10], but with different binding affinities for neurotrophins. High-affinity binding and receptor activation by BDNF is mediated by TrkB. BDNF binding to TrkB results in receptor dimerization and trans-autophosphorylation of tyrosine residues in the carboxyl-terminal intracellular domain that is required for activation of signaling cascades, which include transcriptional activation of the BDNF gene (*BDNF*).[11]

At least three protein isoforms of TrkB are produced by alternative splicing of *NTRK2* pre-mRNAs. These isoforms are the full-length tyrosine kinase receptor, an isoform lacking the tyrosine kinase domain (TrkB-T1), and an isoform lacking the tyrosine kinase domain but containing a Shc binding site (TrkB-T-Shc).[12] All three isoforms are expressed in brain. The T1 isoform has dominant inhibitory effects on BDNF-mediated signaling, although the cellular and molecular functions of truncated TrkB isoforms are not well understood.[13–16] However, there is accumulating evidence that truncated TrkB isoforms have both dominant-negative inhibitory activity on full-length TrkB receptors and yet are capable of mediating signal transduction independent of full-length TrkB receptors.[14,15,17,18]

Evidence supporting a role for BDNF signaling in mechanisms of alcohol and drug dependence has been well documented using different animal models. For example, McGough and co-workers showed that in BDNF-deficient mice, decreased levels of BDNF lead to increased ethanol sensitization and increased voluntary ethanol consumption after a two week withdrawal compared to wild-type animals.[19] In addition, this group showed that when wild-type C57BL/6 mice were allowed to self-administer ethanol, having unlimited access to both water and a 10% ethanol solution for four weeks, *BDNF* mRNA levels were significantly increased in dorsal striatum, a brain region associated with motor control. These results suggest that BDNF signaling is part of regulatory pathway that may offset the development of dependence by limiting alcohol intake. This concept was supported by a previous report where rats received an infusion of BDNF into the ventral tegmental area (VTA), preventing drug-induced adaptations in a brain region known to be involved in drug reinforcing behavior.[20] The essential functional role of BDNF in TrkB-mediated signaling was demonstrated in a model of neurodegeneration, where survival of hippocampal neurons in culture derived from trisomy 16 mice, which produce the truncated kinase deficient isoform, Trk-T1, and would be destined to die were restored by introduction of exogenous full-length TrkB.[21]

A role for TrkB liked to AD in humans has not been reported. In the present study, we found evidence for allele, genotype, and haplotype-based association of *NTRK2* with AD, antisocial personality disorder type, in a Finnish population. These data provide evidence for a new role of TrkB in addictive disorders. As a first approach to identifying sequence variants that may control *NTRK2* expression, we also resequenced the 5′ region of *NTRK2*.

Results

Identification of NTRK2 sequence variants in the 5′ region

Resequencing of the 5′ region of *NTRK2* in a clinically and ethnically diverse sample of 190 unrelated individuals uncovered two novel sequence variants (LNG5 and LNG7; Table 1). The two previously unknown SNPs were located within intronic sequences. Five of the seven variants were previously known and were located in either the 5′ flanking region or 5′-UTR. These other five SNPs were reported in the National Center for Biotechnology Information (NCBI) Single Nucleotide Polymorphism Database (dbSNP) or the Celera Discovery Systems databases. Celera database and NCBI entries are available through the NCBI and the HapMap Project. Preliminary allele frequencies for each SNP were determined from the number of heterozygotes detected by denaturing high-performance liquid chromatography in the screening panel. Variants LNG5 and LNG7 each were detected at an estimated allele frequency of 0.03. This was a preliminary allele frequency based on the entire screening panel, which was composed of individuals from different ethnic populations. Because SNPs LNG5 and LNG7 were previously unknown, we incorporated them into the marker list as SNP2 and SNP4, respectively.

Hardy-Weinberg equilibrium test

Table 2 shows the SNP markers used in the current study. Genotypes were obtained from a total of 43 SNP markers and were tested for meeting Hardy-Weinberg equilibrium (HWE) expectations in the case and control groups, respectively. All SNPs fit HWE in each group ($P > 0.05$) with the exception of SNP 19 ($P = 0.042$) and SNP 32, ($P = 0.045$), which showed marginally significant departures from HWE. These two markers were excluded from subsequent analyses.

Single nucleotide polymorphism association

Genotypes were obtained for each of the 43 SNPs, spanning 363 kb of the *NTRK2* gene (Figure 1, Panel A), and analyzed for allelic and genotypic association. For two markers, strong significant allelic association with AD was observed ($P = 0.0041$ for SNP5 and $P = 0.0019$ for SNP30) (Table 3). SNP 4 and SNP 30 showed genotypic association with AD after Bonferroni correction ($P \leq 0.01$) (Table 3). Of note was the fact that the three significant SNPs (4, 5, and 30) were represented more frequently in the AD group than in the controls, suggesting that these SNPs were associated with AD in the Finnish population.

Linkage disequilibrium of NTRK2 gene

Figure 1 (Panel B) shows pairwise D′ values across the 43 markers in the Finnish control population. D′>0.8 was coded as red. Overall linkage disequilibrium was strong across the 353 kb region. Five LD blocks were observed: block1, located at the 5′ end of *NTRK2*, was defined by eighteen SNPs (SNP1 to SNP18, encompassing the 5′ flanking region and exons 1 through 15 which encode the extracellular and transmembrane domains) covering a physical region of at least 119 kb, accounting for nearly 34% of *NTRK2*. Within block1, more than 85% of SNP pairs showed complete LD (D′>0.99). Ten of the fourteen markers that showed significant association with alcoholism in a single locus analyses were located within this block. Block 2 contained five SNPs (SNP21 to SNP25, encompassing alternatively spliced exon 16). Three SNP pairs were in complete LD within this block. Block 3 contained six SNPs (SNP26 to SNP33), which covered exons 17 to 19. Ten SNP pairs were in complete LD, producing D′ values > 0.99. Block 4 had only two SNPs (SNP 34 and SNP 35, exons 20 to 22) and these markers were in complete LD. Block 5 was composed of seven SNPs from SNP 37 to SNP43, encompassing exons 23 and 24 with 11 SNP pairs in complete LD. Haplotype structure and frequency were estimated within each block separately for case and control groups.

Haplotype-based association with alcohol dependence with antisocial personality disorder

Haplotypes with frequencies greater than 0.03 in each LD block are listed Table 4. In LD block1, eighteen loci generated five haplotypes with frequencies greater than 0.03 and represented 91% of chromosomes in the control group and 86% in the AD group. The global haplotype comparison in block 1 showed a marginally significant difference (global $P = 0.057$). For a single haplotype comparison between control and AD groups, haplotype 1A (which embraced the major allele of SNP4 and SNP5) was represented more frequently in the unaffected control group (0.604) than in the AD group (0.527) ($\chi^2 = 5.898$, $P = 0.013$). The *P*- value remained marginally significant after correction for multiple tests (p = 0.065). Haplotype 1C, which embraced the minor alleles of SNP 4 and SNP5, was more abundant in the AD group (0.129) than in the control group (0.099), although this did not reach statistical significance. Block 2 contained five loci, with five major haplotypes. Haplotype 2B was significantly more abundant in the control group (0.237) than in the AD group (0.181) ($\chi^2 = 4.490$, $P = 0.034$). It was no longer significant after multiple test corrections (corrected $P = 0.170$). There were four, seven-locus, haplotypes in block 3, which accounted for 94% of chromosomes in the controls and 95% of chromosomes in the alcoholic group. Within block 3, haplotype 3A contained the major allele of SNP 30 and was at slightly higher frequency in the control group (0.503) than in AD subjects (0.424) ($\chi^2 = 5.858$, $P = 0.015$, corrected $P = 0.062$), while haplotype 3B, which included the minor allele of SNP 30, was more abundant in the AD group than the control group, supporting the prior single allele association with SNP 30. Haplotype block 4 contained only two loci and generated two major haplotypes (4A and 4B). The frequency of haplotype 4B was 0.246 in the AD group while it was represented at lower frequency (0.192) in the controls ($\chi^2 = 3.988$, $P = 0.046$, corrected $P = 0.092$). Taken together, these results support an association of the *NTRK2* gene with AD, using both single locus and haplotype-based approaches.

Discussion

The current study provides supportive evidence for an association between variation at *NTRK2* and AD. Evidence of association of the *NTRK2* gene with AD was gained using both single locus and haplotype-based approaches. In this population, composed of Finnish Caucasians, three markers, of a total of 43 that were genotyped showed significant genotypic/allelic association with AD. Of note was the fact that two of these markers (SNPs 4 and 5) are located to the 5′ end of *NTRK2*, suggesting that this region may participate in AD directly or indirectly. Moreover, the haplotype-based association results supported over-representation of the 1A haplotype in the control sample, suggesting the presence of a low-risk haplotype in AD.

An additional finding from this study was the pattern of LD across *NTRK2* in a Finnish Caucasian population. Overall linkage disequilibrium was strong across the 353 kb region, and five LD blocks were observed. The HapMap Project generated data on genome-wide levels of variation in different human populations with the intent of guiding the design and analysis of medical genetic studies.[22] We used publicly available genotyping data from HapMap Public Release #20 to compare patterns of LD between the CEU panel (90 individuals [30 trios] from Utah), and the Finnish sample from this study. A total of 511 SNPs from *NTRK2* were genotyped by the HapMap project. Of these, 96 SNPs were selected from HapMap and pairwise measures of LD determined for the *NTRK2* region. Using these values of LD from the CEU panel, we were able to observe a similar block-like structure that was visually apparent in Figure 1B (Q.Y. and K.X., unpublished data). Five LD blocks from the Finnish sample were observed although only 43 SNPs were used in this study, suggesting that the 43 SNPs selected were sufficient to represent the “true” LD structure in this region and that the block structure was not an artifact of either low marker density or marker selection.[23]

To compare haplotype configuration and frequency between CEU and the Finnish samples, we constructed haplotypes using shared SNPs between those used in the HapMap project and this study (26 markers, Table 2). In our analyses, we only considered those haplotypes with a frequency >0.03. We found that haplotype structure and haplotype frequencies were very similar between the two samples ranging from the largest region of LD, Block 1 to the smallest, composed of Block 4. Overall, these results indicate that the block structure and haplotypes within the *NTRK2* region are well conserved between the CEU sample and the Finnish sample. When we initiated this study, HapMap data for the *NTRK2* region was not available, but such cross-comparisons are essential for designing association studies.[23]

It is the long term remodeling of neuronal circuitry that is thought to underlie development of addictive behaviors, including AD.[24] BDNF is well known to be essential for neuronal survival, protection, and activity-dependent synaptic remodeling. BDNF signaling, via TrkB, may alter the expression of target genes related to limiting alcohol of AD.[19] Variation in *NTRK2* affecting expression of full-length or truncated isoforms or their stability could profoundly affect this negative regulatory pathway. Thus, during chronic alcohol exposure, individuals with decreased TrkB-mediated signaling may be at greater risk of becoming AD.

Chronic alcohol exposure has been reported to decrease BDNF mRNA levels in the dentate gyrus and CA1 region of hippocampus[25], although these findings were not supported by subsequent experiments.[26] Global levels and region specific levels of trkB (rat TrkB) mRNA following chronic alcohol exposure were also determined. Levels of trkB mRNA levels were increased in the hippocampus of rats exposed to chronic ethanol, as determined by an RNase protection assay[25], although this was not observed using a semi-quantitative RT-PCR assay.[26] These disparate findings may be due to the fact that different treatment conditions were performed on different rat strains and different detection methods were used for determining BDNF mRNA levels. Future studies are required to refine treatment conditions and expression assays. With these issues in mind, Tapia-Arancibia et al. observed an increase in trkB mRNA levels in the hypothalamus from chronic alcohol exposed rats, a brain region known to be associated with satiety.[25] BDNF-deficient mice exhibited increased eating behavior that was transiently reversed when BDNF was infused.[27] Thus, increased TrkB expression or TrkB-mediated signaling may serve as a negative regulatory pathway involved in food consumption that also acts to regulate ethanol intake. This concept is supported by recent findings showing that *NTRK2* was associated with increased risk to eating disorders and personality traits related to anxiety.[28]

This study has a number of strengths and limitations. We used a relatively large number of SNP markers to capture as much variation as possible across *NTRK2*. Because of this, we were able to perform allele, genotype, and haplotype-based analyses. However, while these association results did not include any known functional variants, they suggest regions of the gene that should have priority in searching for functional variants, particularly the 5′ end of *NTRK2*. In addition, other methodological issues are of concern, where the application of a Bonferroni correction, taking into account the AD phenotype and 43 different markers may be too stringent for detecting the influence of a single gene predicted of having a modest effect in a complex disease such as AD where multiple genes are thought to have an influence. We think that “overcorrecting” the data is a concern justified by the fact that strong LD exists among the different *NTRK2* markers and that the LD blocks themselves are not independent factors, which negates use of a statistical correction. However, as a compromise, we performed a statistical correction by using the number of LD blocks covering this gene for single SNP association tests and presented a global *P*-value, taking into account multiple tests for haplotype-based association.

Regarding clinical characteristics, because 71% of the AD subjects in this study were also diagnosed with antisocial personality disorder (ASPD), there is a possibility that variation at *NTRK2* may also play a role in the course of the disease and not just vulnerability to AD. A plausible functional relationship between alterations in neurotrophin signaling, which would largely occur through TrkB, and comorbid phenotypes with alcoholism has been recently reported by Matsushita et al., who found that the A allele of the G196A (Val66Met) BDNF polymorphism was associated with alcoholic subjects having violent tendencies..[29] However, a smaller study involving 110 AD Chinese convicts with violent histories did not support an association of BDNF Val66Met with ASPD comorbidity.[30] Additional studies will be necessary in different clinical populations to determine if variation at *NTRK2* is associated with ASPD itself, or may modify phenotypes that are comorbid with alcohol dependence.

To our knowledge, this is the first report of *NTRK2* association with AD. Although our initial resequencing effort was confined to the 5′ flanking region, it uncovered two previously unknown SNPs. One of these sequence variants (SNP2) is located between two alternative 5′ UTR exons while the other (SNP4) is located within intron 5, which is 3′ of the first coding exon. Their functional impact on *NTRK2* regulation is not known. As suggested above, future studies will focus on replicating these preliminary findings in other populations, identifying additional sequence variants in affected individuals that may lead to changes in *NTRK2* regulation or TrkB function, and characterization of candidate functional SNPs in different contexts, *in vitro* and *in vivo* in order to better understand how alterations in TrkB expression or function impacts alcohol response-related phenotypes.

Materials and Methods

Screening for sequence variants

To identify potentially functional sequence variants in the 5′ region of *NTRK2*, 3.4 kb of the gene was analyzed in 190 unrelated individuals. The genomic region screened included five know exons in the 5′ untranslated region (5′-UTR) and flanking sequence, comprising 3.1 kb of genomic DNA relative to the translation start site.[12] The “resequencing” sample was composed of 40 African Americans, 40 Finnish Caucasians, and 110 U.S. Caucasians. The sample was also clinically diverse, composed of individuals with anxiety disorders (n = 35), major depression (n = 35), anorexia nervosa (n = 20), obsessive-compulsive disorder (n = 20), ASPD(n = 40), suicidal behavior (n = 40), and alcohol dependence (n =110). DNA samples were obtained from cultured white blood cells from repositories. The resequencing strategy based on high performance liquid chromatography (dHPLC) and direct sequence analysis was described previously.[31] PCR samples with differential dHPLC elution profiles were selected for direct sequence analysis. Typically, the 10 μl sequencing reaction mixture contained 4 μl BigDye Terminator RR Mix (Applied Biosystems, ABI, Foster City, CA); 2.84 μl of dH_2O; 1.6 pmol of forward or reverse primer; and 3 μl of purified PCR amplicon. Cycle conditions for sequencing were 25 cycles consisting of denaturation at 96°C for 10s, annealing at 50°C for 5s, and extension at 60°C for 4 min. Sequencing reaction products were purified by ethanol precipitation, dried, diluted with 25% formamide (v/v), denatured at 95°C for 5 min and analyzed on a 3100 sequencer (ABI, Foster City, CA).

Subjects

The sample Finnish Caucasian population has been described in detail elsewhere.[32] A total of 516 psychiatrically interviewed subjects included 229 alcoholics and 287 healthy controls were recruited under a human research protocol approved by the National Institutes of Health, Bethesda, MD, the University of Helsinki Department of Psychiatry institutional review board, Helsinki, Finland; and the University of Helsinki Central Hospital institutional review board. All subjects provided written informed consent. All subjects were interviewed by two senior

psychiatrists using The Structured Clinical Interview for DSM-III-R (SCID)[33] and were diagnosed using DSM-III-R criteria. The alcohol dependent and alcohol abuse subjects were all male criminal offenders undergoing forensic psychiatric evaluation as inpatients in the Department of Psychiatry, University of Helsinki. Therefore, this population was enriched for antisocial personality disorder (ASPD) and type II alcoholism, which was characterized as alcoholism with antisocial personality disorder.[34] Among 229 cases, 71% individuals were AD with ASPD. The 287 unaffected controls were unrelated healthy Finnish male volunteers recruited through local newspaper advertisements.

Single nucleotide polymorphism (SNP) selection and genotyping

A total of 43 SNP loci from *NTRK2* were selected for genotyping. The markers covered the entire *NTRK2* region with a mean distance of 8796 kb (± 5992 kb) between markers. Genotyping was performed by using 5′- nuclease assays (TaqMan®, Applied Biosystems, Foster City, CA). The allele-specific detection probes and primers for each SNP were designed using Assays-by-Design software (ABI) or were order as Assays-on-Demand. Assay identification numbers are available on request. Approximately 5ng DNA from each sample was plated in 384-well format plates for genotyping with 10% randomly duplicated samples for detecting genotyping error. Genotyping was performed using an Applied Biosystems 7900 Sequence Detector with a 384 well plate format. Three genotype groups were consistently obtained for each biallelic marker based on its relative fluorescence intensity and analyzed by SDS software, version 2.2 (ABI). To access genotype accuracy genotypes were duplicated in approximately 10% of the samples and were randomly selected. Overall genotyping accuracy was 99% with greater than 95% of genotypes completed.

Statistical analyses for linkage disequilibrium, single SNP association, and haplotype-based association

Linkage disequilibrium was estimated based on the formula: D = Pab−PaxPb. D is LD; Pab is the haplotype expectation frequency at loci A and B. The terms Pa and Pb are allele frequencies at two loci. D′ is normalized by the maximum value of possible D. LD was estimated using the Haploview program.[35] If D′ values are ≥ 0.98, there is little evidence for historic recombination. Thus, the two markers are considered to be in "strong LD."[36] An LD block was defined according to Gabriel's definition that less than 5% of comparisons among informative SNP pairs show strong evidence of historic recombine. Five regions of *NTRK2* showed strong LD and were defined as LD blocks.

Genotype and allele frequencies were compared between AD and unaffected control subjects using standard χ^2 tests. Because pairwise LD values were high across five regions of the *NTRK2* region, Bonferroni correction was applied based on five LD blocks. The significance level was set as $p \leq 0.01$. Haplotype structure and frequency within the LD block were inferred separately by case and control groups by means of PHASE, which is a program based on a Bayesian algorithm.[37] The p values of haplotype-based associations for each block were corrected by the number of haplotypes within the block.

Acknowledgements

This study was supported by intramural research grant Z01-AA00325 from the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health (RHL).

References

1. Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, Foroud T, Hesselbrock V, Schuckit MA, Bucholz K, Porjesz B, Li T-K, Conneally PM, Nurnberger JI Jr, Tischfield JA, Crowe RR, Cloninger CR, Wu W, Shears S, Carr K, Crose C, Willig C, Begleiter H. Genome-wide search for genes affecting the risk for alcohol dependence. Am Journal of Med Gen 1998;81:207–215.

2. Li T-K, Hewitt BG, Grant BF. Alcohol use disorders and mood disorders: A National Institute on Alcohol Abuse and Alcoholism perspective. Biol Psychiatry 2004;56:718–720. [PubMed: 15556112]
3. True WR, Xian H, Scherrer JF, Madden PAF, Bucholz KK, Heath AC, Eisen SA, Lyons MJ, Goldberg J, Tsuang M. Common genetic vulnerability for nicotine and alcohol dependence in men. Arch Gen Psychiatry 1999;56:655–661. [PubMed: 10401514]
4. Liu Q-R, Drgon T, Walther D, Johnson C, Poleskaya O, Hess J, Uhl GR. Pooled association genome scanning: Validation and use to identify addiction vulnerability loci in two samples. PNAS 2005;102:11864–11869. [PubMed: 16091475]
5. Bergen A, Yang X, Bai Y, Beerman M, Goldstein A, Goldin L. Genomic regions linked to alcohol consumption in the Framingham Heart Study. BMC Genetics 2003;4:S101. [PubMed: 14975169]
6. Gelernter J, Liu X, Hesselbrock V, Page GP, Goddard A, Zhang H. Results of a genomewide linkage scan: Support for chromosomes 9 and 11 loci increasing risk for cigarette smoking. Amer J Med Genet Part B: Neuropsychiatric Genet 2004;128B:94–101.
7. Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, Bennett PH, Goldman D. Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. Amer J Med Genet 1998;81:216–221. [PubMed: 9603607]
8. Uhl GR, Liu QR, Walther D, Hess J, Naiman D. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. Am J Hum Genet 2001;69:1290–1300. [PubMed: 11704927]
9. Ma J, Zhang D, Dupont R, Dockter M, Elston R, Li M. Mapping susceptibility loci for alcohol consumption using number of grams of alcohol consumed per day as a phenotype measure. BMC Genetics 2003;4:S104. [PubMed: 14975172]
10. Greene LA, Kaplan DR. Early events in neurotrophin signalling via Trk and p75 receptors. Curr Opin Neurobiol 1995;5:579–587. [PubMed: 8580709]
11. Saarelainen T, Vaittinen S, Castren E. TrkB-receptor activation contributes to the kainate-induced increase in BDNF mRNA synthesis. Cell Mol Neurobiol 2001;21:429–435. [PubMed: 11775072]
12. Stoilov P, Castren E, Stamm S. Analysis of the human TrkB gene genomic organization reveals novel TrkB isoforms, unusual gene length, and splicing mechanism. Biochem Biophys Res Commun 2002;290:1054–1065. [PubMed: 11798182]
13. Eide FF, Vining ER, Eide BL, Zang K, Wang X-Y, Reichardt LF. Naturally occurring truncated TrkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. J Neurosci 1996;16:3123–3129. [PubMed: 8627351]
14. Haapasalo A, Koponen E, Hoppe E, Wong G, Castren E. Truncated trkB.T1 is dominant negative inhibitor of trkB.TK+-mediated cell survival. Biochem Biophys Res Commun 2001;280:1352–1358. [PubMed: 11162678]
15. Hartmann M, Brigadski T, Erdmann KS, Holtmann B, Sendtner M, Narz F, Lessmann V. Truncated TrkB receptor-induced outgrowth of dendritic filopodia involves the p75 neurotrophin receptor. J Cell Sci 2004;117:5803–5814. [PubMed: 15507485]
16. Ninkina NAJ, Fischer A, Pinon LF, Buchman VL, Davies AM. Expression and function of TrkB variants in developing sensory neurons. EMBO J 1996;15:6385–6393. [PubMed: 8978665]
17. Baxter GT, Radeke MJ, Kuo RC, Makrides V, Hinkle B, Hoang R, Medina-Selby A, Coit D, Valenzuela P, Feinstein SC. Signal transduction mediated by the truncated TrkB receptor isoforms, trkB.T1 and trkB.T2. J Neurosci 1997;17:2683–2690. [PubMed: 9092589]
18. Steinbeck JAMA. Translational downregulation of the noncatalytic growth factor receptor TrkB.T1 by ischemic preconditioning of primary neurons. Gene Expr 2005;12:99–106. [PubMed: 15892451]
19. McGough NNH, He D-Y, Logrip ML, Jeanblanc J, Phamluong K, Luong K, Kharazia V, Janak PH, Ron D. RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. J Neurosci 2004;24:10542–10552. [PubMed: 15548669]
20. Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (Extracellular Signal Regulated Kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. J Neurosci 1996;16:4707–4715. [PubMed: 8764658]
21. Dorsey SG, Bambrick LL, Balice-Gordon RJ, Krueger BK. Failure of brain-derived neurotrophic factor-dependent neuron survival in mouse Trisomy 16. J Neurosci 2002;22:2571–2578. [PubMed: 11923422]

22. The International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437:1299–1320. [PubMed: 16255080]
23. Phillips MS, Lawrence R, Sachidanandam R, Morris AP, Balding DJ, Donaldson MA, Studebaker JF, Ankener WM, Alfisi SV, Kuo F-S, Camisa AL, Pazorov V, Scott KE, Carey BJ, Faith J, Katari G, Bhatti HA, Cyr JM, Derohannessian V, Elosua C, Forman AM, Grecco NM, Hock CR, Kuebler JM, Lathrop JA, Mockler MA, Nachtman EP, Restine SL, Varde SA, Hozza MJ, Gelfand CA, Broxholme J, Abecasis GR, Boyce-Jacino MT, Cardon LR. Chromosome-wide distribution of haplotype blocks and the role of recombination hot spots. Nature Genet 2003;33:382–387. [PubMed: 12590262]
24. Nestler EJ. Is there a common molecular pathway for addiction? Nat Neurosci 2005;8:1445–1449. [PubMed: 16251986]
25. Tapia-Arancibia L, Rage F, Givalois L, Dingeon P, Arancibia S, Beaugé F. Effects of alcohol on brain-derived neurotrophic factor mRNA expression in discrete regions of the rat hippocampus and hypothalamus. J Neurosci Res 2001;63:200–208. [PubMed: 11169630]
26. Miller R, King MA, Heaton MB, Walker DW. The effects of chronic ethanol consumption on neurotrophins and their receptors in the rat hippocampus and basal forebrain. Brain Res 2002;950:137–147. [PubMed: 12231238]
27. Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. EMBO J 2000;19:1290–1300. [PubMed: 10716929]
28. Ribases M, Gratacos M, Badia A, Jimenez L, Solano R, Vallejo J, Fernandez-Aranda F, Estivill X. Contribution of NTRK2 to the genetic susceptibility to anorexia nervosa, Harm avoidance and minimum body mass index. Mol Psychiatry 2005;10:851–860. [PubMed: 15838534]
29. Matsushita S, Kimura M, Miyakawa T, Yoshino A, Murayama M, Masaki T, Higuchi S. Association study of brain-derived neurotrophic factor gene polymorphism and alcoholism. Alcohol Clin Exp Res 2004;28:1609–1612. [PubMed: 15547445]
30. Tsai S-J, Liao D-L, Yu YW-Y, Chen T-J, Wu H-C, Lin C-H, Cheng C-Y, Hong C-J. A study of the association of (Val66Met) polymorphism in the brain-derived neurotrophic factor gene with alcohol dependence and extreme violence in Chinese males. Neurosci Lett 2005;381:340–343. [PubMed: 15896496]
31. Rudolph JGWS, Sokolsky C, Bozak D, Mazzanti C, Lipsky RH, Goldman D. Determination of melting temperature for variant detection using dHPLC: a comparison between an empirical approach and DNA melting prediction software. Genet Test 2002;6:169–176. [PubMed: 12490056]
32. Lappalainen J, Long JC, Eggert M, Ozaki N, Robin RW, Brown GL, Naukkarinen H, Virkkunen M, Linnoila M, Goldman D. Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. Arch Gen Psychiatry 1998;55:989–994. [PubMed: 9819067]
33. Spitzer, RLWJ.; Gibbon, M.; First, MB. American Psychiatric Press; Washington, DC: 1990. Structured Clinical Interview for DSM-III-R–Non-patient Edition (SCID-NP, Version 1.0).
34. Cloninger C. Neurogenetic adaptive mechanisms in alcoholism. Science 1987;236:410–416. [PubMed: 2882604]
35. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265. [PubMed: 15297300]
36. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The Structure of haplotype blocks in the human genome. Science 2002;296:2225–2229. [PubMed: 12029063]
37. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–989. [PubMed: 11254454]

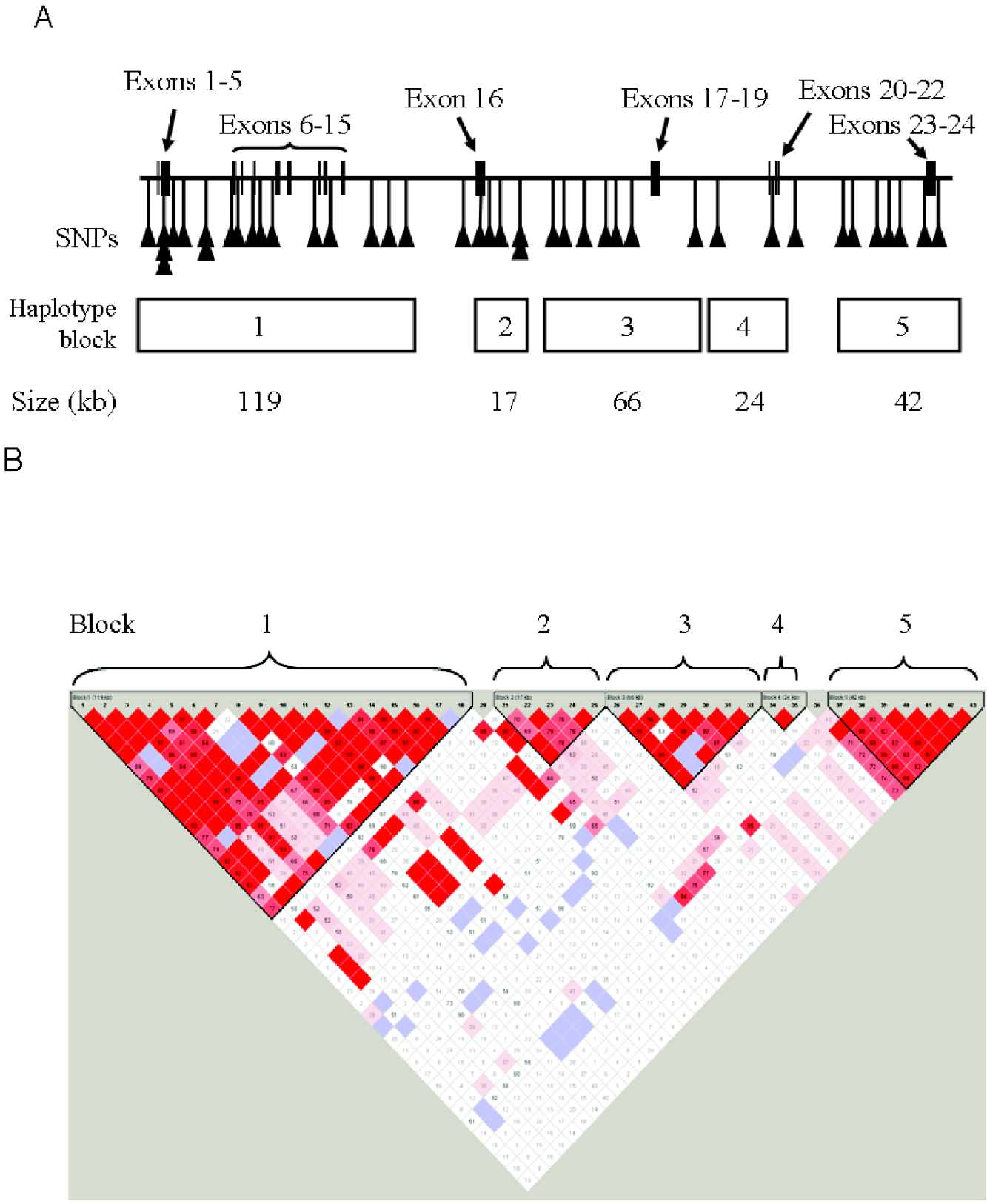

Figure 1.

Panel A, Schematic of the *NTRK2* gene showing locations of the 24 exons, SNP marker positions, and the haplotype blocks with their approximate physical sizes. *Panel B*, Haploview-generated LD map of the *NTRK2* region in the Finnish Caucasian control sample. The AD sample produced similar results. Regions of high LD (D′ = 1 and LOD > 2) are shown in bright red. Markers with lower LD are shown in red (with D′ value indicated as a percentage) through pink (decreasing color intensity indicates decreasing D′ value). Regions with high D′ values (= 1) but low informativeness (LOD < 2) are shown in light blue. Regions of low LD and low LOD scores (D′ < 2, LOD <2) are shown in white. Five haplotype blocks are indicated.

Table 1

Single nucleotide polymorphisms (SNPs) identified or confirmed in the 5′ region of *NTRK2*.

SNP (LNG No.)	Identification Number NCBI	Celera	Variation	Location	Nucleotide position[a]
LNG1	rs1212171	hCV7424025	C>T	promoter	84,512,081
LNG2	rs3758317	hCV3237595	C>T	promoter	84,512,169
LNG3	rs1187321	hCV7424024	A>T	promoter	84,512,585
LNG4	NR[b]	hCV27838439	G>A	promoter	84,513,300
LNG5	NR	NR	A>G	intron 4	84,514,790
LNG6	rs1187325	hCV3237596	G>C	5′-UTR exon 5	84,515,149
LNG7	NR	NR	T>C	intron 5	84,515,471

[a] HapMap NCBI Build 35.

[b] No record in database.

Table 2

NTRK2 single nucleotide polymorphisms (SNPs) used for genotyping.

SNP	Identification Number NCBI	Celera	Variation	Nucleotide position[a]
1*	rs1147198	hCV7424042	C/A	84504902
2 (LNG5)	NR[b]	NR	A/G	84514790
3	rs1187325	hCV3237596	G/C	84515149
4 (LNG7)	NR	NR	T/C	84515449
5*	rs993315	hCV7424005	T/C	84517275
6*	rs1187352	hCV3237603	A/G	84523011
7*	rs1619120	hCV7423969	T/C	84531750
8	NR	hCV3237607	T/C	84533719
9	NR	hCV26567668	C/T	84545393
10	rs7041260	hCV2121698	C/G	84548082
11*	rs1778934	hCV2121705	G/A	84554176
12*	rs10081630	hCV2121711	T/A	84558452
13*	rs2489162	hCV2121715	C/T	84563296
14*	rs3780632	hCV11868468	C/T	84582342
15*	rs7048278	hCV25649147	G/A	84589707
16	rs7027979	hCV11868452	C/G	84610105
17*	rs1573219	hCV7422086	T/C	84617176
18*	rs12555159	hCV7958799	A/G	84624386
19	rs1443444	hCV7958706	C/T	84638865
20	rs3739804	hCV7958645	G/A	84651185
21*	rs1624327	hCV7958636	T/C	84658844
22	NR	hCV7958628	C/T	84664800
23*	rs1036914	hCV7958610	C/T	84667713
24*	rs11140776	hCV1231292	G/T	84676481
25	rs11140777	hCV1231291	C/T	84676515
26*	rs10868229	hCV26567734	G/A	84693116
27*	rs1822420	hCV11923616	T/C	84696830
28*	rs10780690	hCV7958556	T/G	84707726
29*	rs3780634	hCV1231360	G/A	84718272
30*	rs10780691	hCV1231358	C/T	84720807
31	rs11788168	hCV1231354	A/T	84729983
32	rs736744	hCV1231348	A/G	84743961
33*	rs4304401	hCV26566813	T/C	84760065
34*	rs920776	hCV7423761	T/C	84767890
35*	rs1078947	hCV581246	C/T	84792806
36	rs2277192	hCV7958330	G/A	84803531
37	rs10746752	hCV185062	A/G	84825270
38*	rs4504715	hCV185068	A/G	84829632
39*	rs4242630	hCV237788	C/T	84841099
40	rs1565445	hCV1935385	C/T	84846625
41*	rs1490404	hCV1935396	C/T	84852084
42	rs729560	hCV946149	G/A	84863859
43*	rs1490403	hCV1935405	A/T	84868060

[a] HapMap NCBI Build 35.

[b] No record in database.

* In addition to our genotyping results, genotypes and allele frequency data were available for these markers from HapMap Public Release #20 for the Utah sample from the Centre d'Etude du Polymorphisme Humain collection (CEU).

Table 3

Genotype-based and allele-based association with antisocial alcohol dependence[a].

SNP	Phenotype	Genotypes					Allele			
1		11	12	22	Chi Sq	p-value	A1	A2	Chi Sq	p-value
	control	206	64	12	7.224	0.027	476	88	5.3884	0.0203
		73.05	22.7	2.39			0.844	0.156		
	AlcDep	137	73	11			347	95		
		61.99	33.03	4.98			0.785	0.215		
2										
	control	272	9	0	0.694	0.405	553	9	0.3492	0.5546
		96.8	3.2	0			0.984	0.016		
	AlcDep	205	10	0			420	10		
		95.35	4.65	0			0.977	0.023		
3	control	23	109	129	6.912	0.0316	155	367	6.0634	0.0138
		8.81	41.76	49.23			0.297	0.703		
	AlcDep	26	102	77			154	256		
		12.68	49.76	37.56			0.376	0.624		
4	control	12	61	203	10.272	**0.0059**	85	467	5.4735	0.0193
		4.35	22.10	73.55			0.154	0.846		
	AlcDep	8	74	128			90	330		
		3.81	35.24	60.95			0.214	0.786		
5	control	25	114	134	9.059	0.0108	164	382	8.2322	**0.0041**
		9.16	41.76	49.08			0.300	0.700		
	AlcDep	30	107	77			167	261		
		14.02	50.00	35.98			0.390	0.610		
6	control	20	110	158	7.058	0.0293	150	426	5.2354	0.0221
		6.94	38.19	54.86			0.260	0.740		
	AlcDep	19	107	95			145	297		
		8.60	48.42	42.99			0.328	0.672		
7	control	128	121	24	7.937	0.0189	377	169	5.6543	0.0174
		46.89	44.32	8.79			0.690	0.310		
	AlcDep	74	118	24			266	166		
		34.26	56.63	11.11			0.616	0.384		
8	control	276	10	1	1.12	0.5713	562	12	0.0009	0.9754
		96.17	3.48	0.35			0.979	0.021		
	AlcDep	211	10	0			432	10		
		95.48	4.52	0			0.977	0.023		
9	control	271	10	0	0.35	0.5542	552	10	0.1279	0.7206
		96.44	3.56	0			0.982	0.018		
	AlcDep	207	10	0			424	10		
		95.39	4.61	0			0.977	0.023		
10	control	274	10	0	0.341	0.5591	558	10	0.1229	0.7259
		96.48	3.52	0			0.982	0.018		
	AlcDep	210	10	0			430	10		
		95.45	4.55	0			0.977	0.023		
11	control	44	130	108	8.607	0.0135	218	346	6.0673	0.0138
		15.60	46.10	38.3			0.387	0.613		
	AlcDep	42	121	57			205	235		
		19.09	55.00	25.91			0.466	0.534		
12	control	0	12	275	0.193	0.6606	12	562	0.0489	0.825
		0.00	4.18	95.82			0.021	0.979		
	AlcDep	0	11	209			11	429		
		0.00	5.00	95			0.025	0.975		
13	control	40	117	115	8.055	0.0178	197	347	4.1445	0.0418
		14.71	43.01	42.28			0.362	0.638		
	AlcDep	34	117	65			185	247		
		15.74	54.17	30.09			0.428	0.572		
14	control	160	95	23	6.603	0.0368	415	141	3.8463	0.0499
		57.55	34.17	8.27			0.746	0.254		
	AlcDep	101	98	19			300	136		
		46.33	44.95	8.72			0.688	0.312		
15	control	25	94	166	6.661	0.0358	144	426	2.83	0.0925
		8.77	32.98	58.25			0.253	0.747		
	AlcDep	18	97	105			133	307		
		8.18	44.09	47.73			0.302	0.698		
16	control	164	88	24	8.382	0.0151	416	136	3.6417	0.0563
		59.42	31.88	8.7			0.754	0.246		
	AlcDep	100	94	17			294	128		
		47.39	44.55	8.06			0.697	0.303		
17	control	172	82	15	4.3	0.1165	426	112	2.4082	0.1207
		63.94	30.48	5.58			0.792	0.208		
	AlcDep	119	85	12			323	109		
		55.09	39.35	5.56			0.748	0.252		
18	control	270	12	0	0.185	0.6673	552	12	0.0449	0.8322

SNP	Phenotype	Genotypes					Allele			
		95.74	4.26	0			0.979	0.021		
	AlcDep	206	11	0			423	11		
		94.93	5.07	0			0.975	0.025		
20	control	2	36	245	0.912	0.6339	40	526	0.0024	0.9613
		0.71	12.72	86.57			0.071	0.929		
	AlcDep	3	24	194			30	412		
		1.36	10.86	87.78			0.068	0.932		
21	control	179	92	13	2.532	0.282	450	118	2.1369	0.1438
		63.03	32.39	4.58			0.792	0.208		
	AlcDep	124	75	16			323	107		
		57.67	34.88	7.44			0.751	0.249		
22	control	173	94	14	2.387	0.3032	440	122	62.0269	0.200
		61.57	33.45	4.98			0.783	0.217		
	AlcDep	120	80	16			320	112		
		55.56	37.04	7.41			0.741	0.259		
23	control	15	93	163	2.386	0.3033	123	419	2.1467	0.1429
		5.54	34.32	60.15			0.227	0.773		
	AlcDep	18	80	117			116	314		
		8.37	37.21	54.42			0.270	0.730		
24	control	72	134	81	2.587	0.2744	278	296	0.1893	0.6635
		25.09	46.69	28.22			0.484	0.516		
	AlcDep	45	119	59			209	237		
		20.18	56.36	26.46			0.469	0.531		
25	control	77	135	72	2.217	0.3301	289	279	0.5435	0.461
		27.11	47.54	25.35			0.509	0.491		
	AlcDep	59	116	44			234	204		
		26.94	52.97	20.09			0.534	0.466		
26	control	59	135	78	6.864	0.0323	253	291	6.4835	0.0109
		21.69	49.63	28.68			0.465	0.535		
	AlcDep	66	100	45			232	190		
		31.28	47.39	21.33			0.550	0.450		
27	control	3	73	211	1.165	0.5585	79	495	0.8197	0.3653
		1.05	25.44	73.52			0.138	0.862		
	AlcDep	4	63	155			71	373		
		1.80	28.38	69.82			0.160	0.840		
28	control	63	151	59	7.345	0.025	277	269	3.9322	0.0474
		22.26	53.36	24.38			0.507	0.493		
	AlcDep	68	116	36			252	188		
		30.91	52.73	16.36			0.573	0.427		
29	control	206	44	2	0.556	0.7575	456	48	0.2195	0.6395
		81.75	17.46	0.79			0.905	0.095		
	AlcDep	165	38	3			368	44		
		80.10	18.45	1.46			0.893	0.107		
30	control	66	142	62	11.707	**0.0029**	274	266	9.6288	**0.0019**
		24.44	52.59	22.96			0.507	0.493		
	AlcDep	26	111	65			163	241		
		12.87	54.95	32.18			0.403	0.597		
31	control	259	14	0	0.076	0.7823	532	14	0.0042	0.9483
		94.87	5.13	0			0.974	0.026		
	AlcDep	208	10	0			426	10		
		95.41	4.59	0			0.977	0.023		
33	control	270	15	0	0.756	0.3846	555	15	0.418	0.5179
		94.74	5.26	0			0.974	0.026		
	AlcDep	212	8	0			432	8		
		96.36	3.64	0			0.982	0.018		
34	control	180	98	5	7.187	0.0275	458	108	4.9379	0.0263
		63.60	34.63	1.77			0.809	0.191		
	AlcDep	121	86	12			328	110		
		55.25	39.27	5.48			0.749	0.251		
35	control	8	105	166	2.913	0.2331	121	437	2.204	0.1377
		2.87	37.63	59.5			0.217	0.783		
	AlcDep	11	90	115			112	320		
		5.09	41.67	53.24			0.259	0.741		
36	control	6	74	206	3.461	0.1772	86	486	0.659	0.4169
		2.10	25.87	72.03			0.150	0.850		
	AlcDep	2	71	146			75	363		
		0.91	32.42	66.67			0.171	0.829		
37	control	222	62	4	2.338	0.3107	506	70	0.9898	0.3198
		77.08	21.53	1.39			0.878	0.122		
	AlcDep	159	60	2			378	64		
		71.95	27.15	0.9			0.855	0.145		
38	control	197	65	12	1.813	0.4039	459	89	0.0997	0.7522
		71.90	23.72	4.38			0.838	0.162		
	AlcDep	157	57	5			371	67		
		71.69	26.03	2.28			0.847	0.153		

SNP	Phenotype	Genotypes					Allele			
39	control	92	147	41	2.709	0.2581	331	229	2.1819	0.1396
		32.86	52.50	14.64			0.591	0.409		
	AlcDep	60	112	42			232	196		
		28.04	52.34	19.63			0.542	0.458		
40	control	41	144	98	2.761	0.2514	226	340	2.3488	0.1254
		14.49	50.88	34.63			0.399	0.601		
	AlcDep	42	112	64			196	240		
		19.27	51.38	29.36			0.450	0.550		
41	control	40	142	98	2.26	0.3231	222	338	1.6882	0.1938
		14.29	50.71	35			0.396	0.604		
	AlcDep	41	107	67			189	241		
		19.07	49.77	31.16			0.440	0.560		
42	control	41	147	93	2.01	0.366	229	333	1.085	0.2976
		14.59	52.31	33.1			0.407	0.593		
	AlcDep	42	108	67			192	242		
		19.35	49.77	30.88			0.442	0.558		
43	control	40	147	94	1.402	0.4961	227	335	0.7199	0.3962
		14.23	52.31	33.45			0.404	0.596		
	AlcDep	38	105	66			181	237		
		18.18	50.24	31.58			0.433	0.567		

[a]Genotype and allele counts are shown for each phenotype group. The numbers below each count are frequencies, given as per cent. *P*-values <0.01 (after multiple test correction) are shown in bold font.

Table 4

Haplotype-based association with antisocial alcohol dependence.

Block	Haplotype	Control (N=287)	AD (N=222)	Global *P*	*P**	Corrected *P*
1A	112222111122212111	0.604	0.527	0.057	0.013	0.065
1B	111211211112112111	0.127	0.141			
1C	211111211112121221	0.099	0.129			
1D	112222211112121221	0.046	0.026			
1E	112222111112121221	0.037	0.037			
2A	11221	0.485	0.512			
2B	11212	0.237	0.181	0.944	0.034	0.170
2C	22112	0.161	0.214			
2D	21212	0.046	0.036			
2E	12112	0.039	0.032			
3A	2221111	0.503	0.424	0.740	0.015	0.062
3B	1211211	0.238	0.283			
3C	1111211	0.131	0.160			
3D	1212211	0.066	0.087			
4A	12	0.784	0.739			
4B	21	0.192	0.246	0.862	0.046	0.092
5A	1112222	0.561	0.522			
5B	1121111	0.137	0.162			
5C	1221111	0.145	0.134			
5D	2121111	0.114	0.141			

* *P*- value was calculated for each individual haplotype.